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MOLECULAR STUDY OF EXTEND SPECTRUM β -LACTAMASES AMONG EXTRAINTESTINAL *ENTEROBACTER CLOACAE* RECOVERED FROM PATIENTS WITH CAUTI, HILLA-IRAQ

HUSSEIN OLEIWI MUTTALEB AL-DAHMOSHI
Faculty of Science, Department of Biology, Babylon University, Iraq

ABSTRACT

Background: *Enterobacter cloaca* is important nosocomial pathogens responsible for various infections including catheter associated urinary tract infections (CAUTIs) and lower respiratory tract infections. It can be assigned to one of main four phylogenetic groups including intestinal (A and B1) groups and extraintestinal (B2 and D) groups. Many (ESBLs) can be found in *E. cloacae* like $bla_{TEM,}$ $bla_{CTX\text{-}M}$, bla_{SHV} and $bla_{OXA.}$ It was also have the ability to form biofilm and rendering them resistant to antimicrobials and host defenses.

Material: Uriscan test (using urine strip) was used to detect pyuria among urine samples. All samples were cultured on routine culture medium and the suspected isolates confirmed with automated diagnosis with Viteck 2 Compact system and then by conventional PCR for *16s rRNA* gene for *E. cloacae*. The confirmed *E. cloacae* isolates investigated genotepically to identify the phylogenetic group and only extraintestinal *E. cloacae* isolates will screened for ESBLs phenotypes (using DDST) and the confirmed by genotypic assessments for ESBLs production.

Results: Among 120 urine samples only 53 (44.16%) of samples positive for culture (Bacteriuria) among which 7 (13.20%) isolates (from different urine samples), were phenotypically and genotepically identified as *E. cloacae.* Five isolates of *E. cloacae* were biofilm former. The results of phylogenetic analysis revealed that only two isolates belong to intestinal phylogenetic groups (group A and B1) while the rest, five isolates, belongs to extra intestinal groups (four isolates belong to group B2 and single isolates belong to group D). The phenotypic investigation of ESBLs display that, two isolates were negative for of ESBLs while five isolates were positive. The genotypic investigation of ESBLs results revealed that (3/7) *E. cloacae* isolates carrying $\boldsymbol{bla_{TEM}}$, $\boldsymbol{bla_{SHV}}$, $\boldsymbol{bla_{CTX\text{-}M}}$ genes.

Conclusions: Although *E. cloacae* was intestinal opportunistic pathogen, but can cause serious extraintestinal infections like CAUTI which may be still untreatable due to their possessing of differ types of ESBLs like $\boldsymbol{bla_{TEM}}$, $\boldsymbol{bla_{SHV}}$, $\boldsymbol{bla_{CTX\text{-}M.}}$

KEYWORDS: *E. cloacae*, Phylogeny, Pyuria, Biofilm, ESBLS

INTRODUCTION

Catheter associated urinary tract infection (CAUTI) is the type of UTIs that occur due to inserted urinary catheter via urethra for long time [1]. The home of microorganisms causing CAUTI can be endogenous, may be by way of rectal, or vaginal colonization, or exogenous, such as via unclean hands of healthcare employees or equipment. The pathogens can come in the urinary tract either by the extraluminal route, via passage along the outside of the catheter in the periurethral mucous sheath, or by the intraluminal route, via migration along the internal lumen of the catheter from a contaminated collection bag or catheter-drainage tube junction [2].

Extended-spectrum beta-lactamases (ESBLs) are enzymes that confer resistance to extended-spectrum cephalosporins within the family Enterobacteriaceae. It represents an ever-growing class of plasmid-mediated β-lactamase found in Gram-negative bacilli. They are associated with broad-spectrum resistance to penicillins, cephalosporins, and aztreonam. They arise from point mutations within genes for plasmid-mediated beta-lactamases such as TEM-1, TEM-2 and SHV-1 [3].

Over 150 different mutations have been described. They are commonly encoded on large transferable plasmids which also encode resistance to other antibiotic classes, therefore often express high levels of co-resistance to aminoglycosides, quinolones, β-lactam/β-lactamase-inhibitor combinations and co-trimoxazole. These genes can be transferred concurrently from strain to strain with the implication that co-selection can occur where resistance to two different classes arises by the use of either agent [4].

Most of the microbes responsible for urinary tract infection (UTI) have the capacity to produce ESBLs in huge quantities, which are normally present in the intestinal flora without damaging the host. Usually ESBLs are plasmid borne and confer multiple drug resistance, creating problems in treating UTI [5].

Gram-negative bacteria that contain group 2be extended spectrum beta-lactamases (ESBLs) and group 1 AmpC cephalosporinases are a growing worldwide concern [6]. These organisms not only produce potent enzymes that render them resistant to beta-lactam antibiotics, but they are frequently resistant to many other classes of antibiotics [7].

An *Enterobacter cloaca* is important nosocomial pathogens responsible for various infections. They colonize the gastrointestinal tract and are an important cause of nosocomial and opportunistic infections. Wide range of Extraintestinal infections can be caused by Enterobacter species including bacteremia, catheter associated urinary tract infections (CAUTIs), lower respiratory tract infections, endocarditis, intra-abdominal infections, septic arthritis, osteomyelitis, and ophthalmic infections [8].The source of infection may be endogenous (via colonization of the skin, gastrointestinal tract, or urinary tract) or exogenous, resulting from the ubiquitous nature of Enterobacter species. However, data from isolates recovered from the ICU revealed that *Enterobacter cloaca* regard the fifth most common pathogens recovered from the urinary tract [9]. Urinary Tract Infections (UTIs), including cystitis and pyelonephritis, are among the most frequent human extraintestinal infections. The clinical manifestations of urinary tract infections due to *Enterobacter* spp. differ little from those of infections due to other Gram-negative bacilli [10].

Phylogeny is the study of evolutionary relatedness among various groups of organisms. Molecular phylogeny has also revealed that horizontal transfer plays an important and unexpected role in evolution [11]. Phylogenetic classification is concerned with grouping individual species into evolutionary categories. Besides phylogenetic classification has been made much more facile by the invention of molecular taxonomy, based on the nucleotide sequence divergence at individual loci (genes) [12].

Phylogenetic analysis has shown that *Escherichia coli* strains can be assigned to one of the main phylogenetic groups (A, B1, B2, and D) [13]. The extraintestinal pathogenic strains usually belong to groups B2 and D [14], the commensal strains to groups A and B1, whilst the intestinal pathogenic strains belong to groups A and B1 [15]. Clermont et al. (2000) [16] have developed a PCR based method to characterize the phylo-groups using the genetic markers *chuA*, *yjaA* and the DNA fragment *TspE4.C2*. Most of the of *Enterobacter cloacae* isolates belong to the Extraintestinal phylogenetic groups especially B2 [17]. Abdul-Razzaq et.al. (2013)[17] demonstrate that Phylogenetic

group B2 (especially subgroup $B2_3$) was predominant among *Enterobacter* spp. isolates recovered from patients with cystitis. Our study aimed to detects the ESBLs producing *Enterobacter cloacae* isolates recovered from patients with (CAUTIs).

MATERIALS AND METHODS

Patients

One hundred twenty urine samples were recovered from patients with urinary catheter admitted to Al-Hilla teaching hospital, Hilla-Iraq. All patients were staying for more than 5 days with mean age (44.3 years). Each urine sample was cultured on MacConkey agar, EMB agar, ODC broth and LDC broth and continued with traditional biochemical tests and PCR technique to confirmed the identification.

Bacterial Diagnosis

All suspected *E. cloacae* isolates were screened with biochemical test and then confirmed with automated diagnosis with Viteck 2 Compact system (Biomeruex/France) and then confirmed by conventional PCR for *16s rRNA* gene for *E. cloacae*. The confirmed *E. cloacae* isolates were investigated genotepically to identify the phylogenetic group using three genetic markers chuA, yjaA and the DNA fragment TspE4.C2. Finally, only extraintestinal *E. cloacae* isolates were screened for ESBLs phenotypes (using DDST) and the confirmed by genotypic assessments for ESBLs production [16, 17, 19].

Biofilm Formation Assay

For biofilm detection the Tissue culture plate (TCP) assay (also called semi quantitative microtiter plate test) described by Christensen *et al*., (1985)[20] was used for detection of biofilm formation with some modification as follow: Isolates from fresh agar plates were inoculated in TSB containing 1% glucose and incubated for 18 hour at 37°C and then diluted 1:100 with fresh TSB. Individual wells of sterile, polystyrene, 96 well-flat bottom tissue culture plates' wells were filled with 150 µl aliquots of the diluted cultures and only broth served as control to check non-specific binding of media. Each isolate was inoculated in triplicate. The tissue culture plates were incubated for 24 hours at 37°C. The next step includes quietly removing of content of each well by tapping the plates. The wells then washed away four times with phosphate buffer saline (PBS pH 7.2) to remove free-floating 'planktonic' bacteria. Adherent 'sessile' organisms (Biofilm) in plate were fixed by placing in oven at 37°C for 30 min. All wells stained with crystal violet (0.1% w/v). Excessive stain was eroded by thorough washing with deionized water and then kept for drying. Adding of 150 µl of acetone/ethanol (20:80, v/v) mixture to dissolve bounded crystal violet. Read the optical density (O.D.) at 630 nm (triplicate for each sample, means three readings for each sample).

Phenotypic Investigation of ESBLs

The detection of ESBL phenotype among *E. cloacae* isolates was performed according to (2012)[21]using double-disc synergy test (initial screening test and confirmatory test). In this test four system were used represented by: (cefpodoxime + cefpodoxime-clavulanic acid), (cefotaxime + cefotaxime-clavulanic acid), (ceftazidime + ceftazidime-clavulanic acid) and (ceftriaxone + ceftriaxone-clavulanic acid) (Himedia / India)

DNA Extraction form *E. cloacae*

Bacterial DNA was extracted from the *Enterobacter* spp. isolates according to protocol provided by manufacturer using Wizard Genomic DNA purification kit supplemented by (Promega, USA). The extracted DNA was checked by 0.7% agarose gel electrophoresis and viewed using UV-transilluminator [22].

Detection of Phylogenetic Groups and ESBLs Genes by PCR

Conventional PCR was achieved to investigate *16s rRNA* gene for *E. cloacae* to confirm phenotypic identification [23]. PCR was also conducted to determine the phylogenetic grouping of the isolates by targeting two genes, *chuA*, *yjaA* and anonymous DNA fragment *TspE4.C2* [16]. Also the bacterial DNA were used as templates in the specific PCR amplifications for detection of the *blaTEM, blaSHV, blaCTX-M, blaIBC, blaVEB, blaSFO, blaPER, blaOXA-3, blaOXA-4*, genes [24, 25, 26, 27]. The single PCR reaction (25 μl) mixture consist of 3 μl of upstream primer, 3 μl of downstream primer, 9 μl of free nuclease water, 5 μl of DNA and 5 μl of master mix powered in 0.2 ml thin walled PCR tube. The Multiplex PCR reaction (25 μl) mixture consist of 2-2.5 μl of upstream primer, 2-2.5 μl of downstream primer, 3-5 μl of free nuclease water, 5 μl of DNA and 5 μl of master mix powered in 0.2ml thin walled PCR tube. Thermal cycler used in this study was (Clever Scientific / UK). The Thermal cycler conditions for phylogenetic groups' markers were as follows: 94°C for 5 min followed by 30 cycles of 94°C for 30 s, 59°C for 30 s and 72°C for 30 s. A final extension of 72°C for 7 min was performed at the end of PCR. The primers used were *chuA*, *yjaA* and *TspE4.C2* which generated 279, 211 and 152 bp fragments respectively [16]. The Thermal cycler conditions for ESBLs genes markers were as follows: 94°C for 5 min followed by 30 cycles of 94°C for 30 s, (42°C for 30 s for *16s rRNA* gene; 42°C for 30 s for *blaPER, blaOXA-3; blaOXA-4* ; and 47°C for 30 s for *blaSHV* ; 55°C for 30 s for each of *blaSHV, blaCTX-M and blaTEM* ; 46°C for 30 s for *blaVEB*; 47°C for 30 s for *blaIBC*; 44°C for 30 s for *blaSFO*) and 72°C for 30 s. A final extension of 72°C for 7 min was performed at the end of PCR [23, 24, 25, 26, 27]. The multiplex mixtures were done as follow: (*blaTEM + blaCTX-M + blaSHV*), (*blaPER + blaOXA-3 + blaOXA-4*),

RESULTS

Among 120 urine samples collected from patients with urinary catheter, only 53(44.16%) of urine samples positive for culture (Bacteriuria) figure 1, among which 7 (13.20%) isolates (from different urine samples), were phenotypically and genotepically identified as *E. cloacae*. The remaining isolates represented by 29 (54.72%) isolates of *E. coli,* 9 (16.98%) isolates of *K. pneumonia,* 6 (11.32%) isolates of *P. vulgaris* and 2 (3.77%) isolates of *E. aerogenes* figure 2.

The confirmation of the *E. cloacae* isolates diagnosis were done specific primer pair target the *16s rRNA* sequence and the results revealed that, all the suspected (7 isolates isolates) were *E. cloacae* as depicted in the figure 3. Relating to the results of biofilm formation, tissue culture plate (TCP) assay revealed that five isolates of *E. cloacae* were biofilm former while the rest (two isolates) were non biofilm former figure 4.

Concerning the phylogenetic analysis of *E. cloacae*, table 3 summarize the groups according to the results gathered from figure 5, 6 and 7. The results revealed that only two isolates belong to intestinal phylogenetic groups (group A and B1) while the rest, five isolates, belongs to extraintestinal groups (four isolates belong to group B2 and single isolates belong to group D). The results of double-disc synergy test (initial screening test and confirmatory test) revealed that two isolates were negative for phenotypic investigation of ESBLs production while five isolates were positive table 4.

The genotypic investigation of ESBLs genes among *E. cloacae* isolates include the detection of the *blaTEM, blaSHV, blaCTX-M, blaIBC, blaVEB, blaSFO, blaPER, blaOXA-3, blaOXA-4*, genes. All isolates gave negative results for *blaSHV, blaIBC, blaVEB, blaSFO, blaPER, blaOXA-3* and *blaOXA-4*, genes table 5. Three isolates were positive for each of *blaTEM* and *blaCTX-M* while two isolates were positive for *blaSHV* as shown in figure 8.

DISCUSSIONS

Among CAUTI patients, the Bacteriuria compile 53(44.16%), the other causes of CAUTI consist 47(55.84%) and may be attributed to other microbes like *Candida spp.* or may be due to viral causes. The percentage of the bacterial causes was as follow: 29 (54.72%) isolates of *E. coli,* 9 (16.98%) isolates of *K. pneumonia,* 7 (13.20%) isolates were phenotypically and genotepically identified as *E. cloacae,* 6 (11.32%) isolates of *P. vulgaris* and 2 (3.77%) isolates of *E. aerogenes.* Our results approximately similar to those gathered by Hidron *et. al.* (2008)[28] who report that most frequent pathogens associated with CAUTI in hospitals reporting to NHSN between 2006-2007 were *Escherichia coli* (21.4%) and Candida spp (21.0%), followed by *Enterococcus* spp (14.9%), *Pseudomonas aeruginosa* (10.0%), *Klebsiella pneumoniae*(7.7%), and *Enterobacter* spp (4.1%). A smaller proportion was caused by other gram-negative bacteria and *Staphylococcus* spp [28]. CAUTI is rarely they may be due to viral or fungal infections [29].

When assessing the need for a urinary catheter, the risk of catheter associated urinary tract infection (CAUTI) should be considered. The introduction of a catheter into the bladder circumvents the body's normal defense mechanisms and enables micro-organisms to track up the external catheter surface into the bladder [30]. Once a catheter is *in situ*, bacteria in the urinary drainage bag or introduced via the catheter/bag's connection points create biofilms on the surface of the catheter lumen, which are a precursor to CAUTI [31].

The daily risk to catheterized patients of developing bacteriuria is 3-6% and cumulatively increases the longer the catheter remains in place. Consequently, around 50% of hospitalised patients catheterised for longer than 7-10 days develop bacteriuria. Although patients with bacteriuria frequently do not present with clinical symptoms, 20-30% of them will develop symptoms of CAUTI. Many of these infections are serious and lead to significant morbidity and mortality [32].

Concern biofilm formation, the results display that, five of seven isolates of *E. cloacae* regards as strong former of biofilm. Formation of biofilms by urinary pathogens on the surface of the catheter and drainage system occurs universally with prolonged duration of catheterization. Over time, the urinary catheter becomes colonized with microorganisms living in a sessile state within the biofilm, rendering them resistant to antimicrobials and host defenses and virtually impossible to eradicate without removing the catheter. The role of bacteria within biofilms in the pathogenesis of CAUTI is unknown and is an area requiring further research [33].

The tissue culture plate test (TCP) was designated in the literature as a simple and rapid method to quantify biofilm formation of different bacterial strains [34]. The basic dye (Crystal violet) bind to negatively charged molecules on the cell surface as well as nucleic acids and polysaccharides, and hence gives an overall measure of the whole biofilm. It has been used as a regular technique for rapidly gain access to cell attachment and biofilm formation in a range of Gram-negative [35].

Regarding the phylogenetic analysis of *E. cloacae* isolates, the results showed that the *chuA* gene was present in all isolates recovered from urine belonging to groups B2 and D and was absent from all isolates belonging to groups A and B1 [36, 37]. The *yjaA* gene allowed perfect discrimination between group B2 and group D (found in all Group B2 isolates and absents in Group D isolates) and it was present in all isolates belonging to Subgroup A1 isolates and absent in all isolates of group B1. Also, the *TspE4.C2* is present in group B1 isolates, group B2 (only Subgroup B23) and group D (Subgroup D2) and absent from all group A isolates [38]. Alteri and Mobley (2007)[39] displayed that, *ChuA* is most important outer membrane proteins significantly induced in human urine as iron compound receptors. These findings showed that human urine is an iron-limiting environment and concurrent production of numerous iron compound receptors by uropathogen may represent a fundamental strategy for the ability of this pathogen to colonize the human urinary tract. Many studies reveal that *chuA* was acquired by sister groups B2 and D soon after their emergence rather than being present in common ancestor and subsequently being lost by group B1 and group D [40].

The results gathered from this study demonstrate that, among urine sample isolates the extraintestinal groups isolates were dominant while occurrence of intestinal groups was very low. This result agreed with many studies like Johnson et al., (2001)[41] and Zhao et al., (2009)[42] who found that the UPEC isolates primarily belonged to one of two virulence groups group B2 or D. Predominance of B2 group among extraintestinal groups isolated from urine was in agreement with many studies [17,43].

Although ESBLs have been reported at a much lower frequency among chromosomal AmpC β-lactamase-producing members of the family Enterobacteriaceae, such as Enterobacter spp., Citrobacter spp., and Serratia spp. [44], the prevalence of ESBL in these species is increasing. TEM-type ESBLs have been reported in other genera of Enterobacteriaceae such as *Enterobacter aerogenes*, *Enterobacter cloacae* [45]. The native TEM-1 β-lactamase confers resistance to ampicillin, penicillin and first-generation cephalosporins such as cephalothin. SHV-type ESBLs was also found in Enterobacter spp.

Spanu et al.,(2002)[46] found that (35%), (45%) and (3%) of E. aerogenes isolates express *TEM* type only, *SHV* type only and *TEM* and *SHV* ESBLs respectively while (25%), (25%) and (50%) of *E. cloacae* isolates express *TEM* type only, *SHV* type only and *TEM* and *SHV* ESBLs respectively.*VEB-1* has also been found in *Enterobacter* spp. [47]. *CTX-M-10* and *CTX-M-25* ESBLs were expressed by *E. aerogenes while* only *CTX-M-2* and *SHV-12* expressed by *E. cloacae* [48]. *CTX-M* ESBLs are the most prevalent ESBLs worldwide. Recently, *CTX-M* ESBLs have been reported in Egypt, with *CTX-M-15* being the most common ESBL reported in the Middle East region and North Africa [49, 50]. *CTX-Ms* are class A ESBLs that are most active against cefotaxime. However, some *CTX-Ms* can hydrolyze ceftazidime such as *CTX-M-15* and *CTX-M-19* [51]. This study conclude that although *E. cloacae* was intestinal opportunistic pathogen, but can cause serious extraintestinal infections like CAUTI which may be still untreatable due to their possessing of differ types of ESBLs like blaTEM, blaSHV, blaCTX-M.

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APPENDICES

Table 1: Primer Pairs of Phylogenetic Groups

Genes	Primer Sequence (5'-3')	Size (bp)	References
chuA	F: GACGAACCAACGGTCAGGAT R: TGCCGCCAGTACCAAAGACA	279	[14]
yjaA	F: TGAAGTGTCAGGAGACGCTG R: ATGGAGAATGCGTTCCTCAAC	211	
TspE4C2	F: GAGTAATGTCGGGGCATTCA R:CGCGCCAACAAAGTATTACG	152	

Table 2: Primer Pairs of ESBLs Genes

Genes	Primer Sequence (5'-3')	Size (bp)	References
16S	F: CCGCACAAGCGGTGGAGCA R: AGGCCCGGGAACGTATTCAC	400	[21]
bla_{OXA-3}	F:ACACAATACATATCAACTTCGC R:AGTGTGTTTAGAATGGTGATC	700	[22]
bla_{OXA-4}	F: ACACAATACATATCAACTTCGC R: AGTGTGTTTAGAATGGTGATC	810	
bla_{SHV}	F: ATGCGTTATATTCGCCTGTG R:AGATAAATCACCACAATGCGC	780	[23]
bla_{CTX-M}	F: CGYTTTSCIATGTGCAG R: ACCGCRATATCRTTGGT	550	
bla_{IBC}	F: GGGCGTACAAAGATAATTTCC R: GAAGCAACGTCGGCTTGAACG	940	

Table 2: Contd.,

bla_{VEB}	F: ACGGTAATTTAACCAGATAGG R: ACCCGCCATTGCCTATGAGCC	970	
bla_{SFO}	F: GTTAATCCATTTTATGTGAGG R: CAGATACGCGGTGCATATCCC	940	
bla_{PER}	F: ATGAATGTCATTATAAAAGC R: AATTTGGGCTTAGGGCAGAA	930	[24]
bla_{TEM}	F: TTC TTG AAG ACG AAA GGG C R: ACG CTC AGT GGA ACG AAA	1207	[25]

F, forward primer; R, reverse primer. R, A or G; M, A or C; Y, C or T; K, G or T; S, G or C; I, Inosin

Table 3: Distribution of Phylogenetic Markers among *E. cloacae* Isolates

Isolate No.	*chuA*	*yjaA*	*TspE4.C2*	Phylogroup
E. cloacae 1	+	+	-	Extraintestinal Group B2
E. cloacae 2	-	-	-	Intestinal Group A
E. cloacae 3	-	+	+	Intestinal Group B1
E. cloacae 4	+	+	+	Extraintestinal Group B2
E. cloacae 5	+	-	-	Extraintestinal Group D
E. cloacae 6	+	+	+	Extraintestinal Group B2
E. cloacae 7	+	+	+	Extraintestinal Group B2

Table 4: Results of Double-Disc Synergy Test (Initial Screening Test and Confirmatory Test)

Isolate No.	Initial Screen Test Zone Diameter (mm)				Phenotypic Confirmatory Test Zone Diameter (mm)				ESBLs production
	Cefpodoxime (10µg)	Cefotaxime (30µg)	Ceftazidime (30µg)	Ceftriaxone (30µg)	Cefpodoxime-clavulanic acid (10/10µg)	Cefotaxime-clavulanic acid (30/10µg)	Ceftazidime-clavulanic acid (30/10µg)	Ceftriaxone-clavulanic acid (30/10µg)	
E. cloacae 1	19	29	25	27	20	30	26	30	Negative
E. cloacae 2	15	25	20	29	21	29	27	33	Positive
E. cloacae 3	20	31	24	32	20	30	25	32	Negative
E. cloacae 4	14	22	22	22	20	29	28	25	Positive
E. cloacae 5	17	26	22	26	23	33	26	27	Positive
E. cloacae 6	14	23	19	28	21	29	26	27	Positive
E. cloacae 7	16	28	17	23	23	35	24	26	Positive

Table 5: Distribution of ESBLs Genes among *E. cloacae* Isolates

Isolate No.	bla_{TEM}	bla_{SHV}	bla_{CTX-M}	bla_{IBC}	bla_{VEB}	bla_{SFO}	bla_{PER}	bla_{OXA-3}	bla_{OXA-4}	Genotype
E. cloacae 1	_	_	_	_	_	_	_	_	_	_
E. cloacae 2	_	_	_	_	_	_	_	_	_	_
E. cloacae 3	_	_	_	_	_	_	_	_	_	_
E. cloacae 4	_	_	_	_	_	_	_	_	_	_
E. cloacae 5	+	+	+	_	_	_	_	_	_	bla_{TEM}^{+}, bla_{SHV}^{+}, bla_{CTX-M}^{+}
E. cloacae 6	+	+	_	_	_	_	_	_	_	bla_{TEM}^{+}, bla_{SHV}^{+}
E. cloacae 7	+	+	_	_	_	_	_	_	_	bla_{TEM}^{+}, bla_{SHV}^{+}

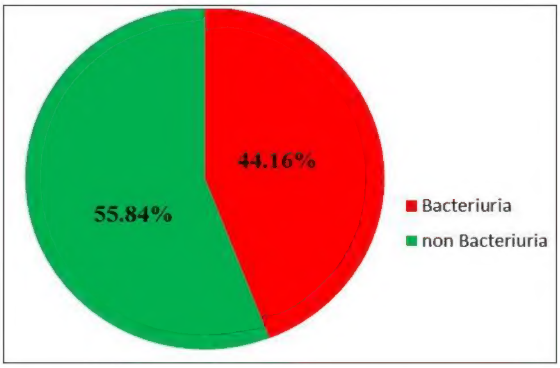

Figure 1: Distribution of Bacteriuria among CAUTI Patients

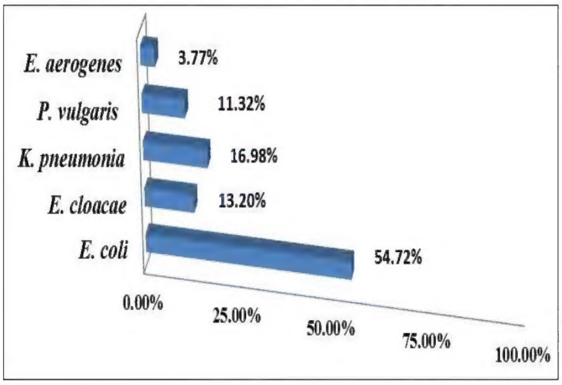

Figure 2: Distribution of Bacterial Isolates among CAUTI Patients

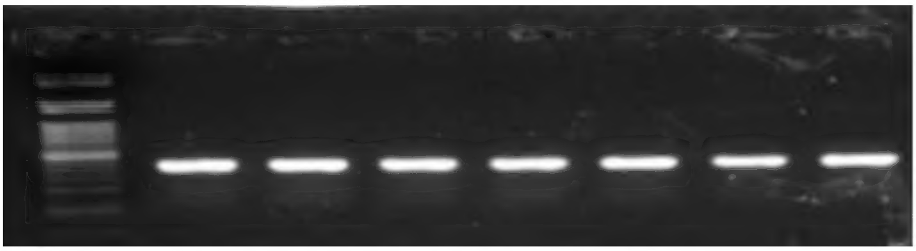

Figure 3: 2% Agarose Gel Electrophoresis of PCR Products of *16s rRNA* Gene (400 bp). Lane 1 Represent Ladder (100 bp), Lane 2-8 Represent *E. cloacae* Isolates

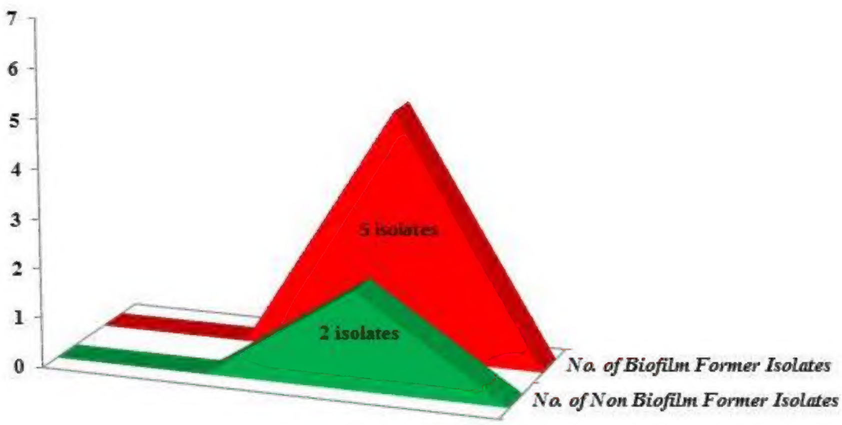

Figure 4: Distribution of *E. cloacae* Isolates According to Biofilm Formation

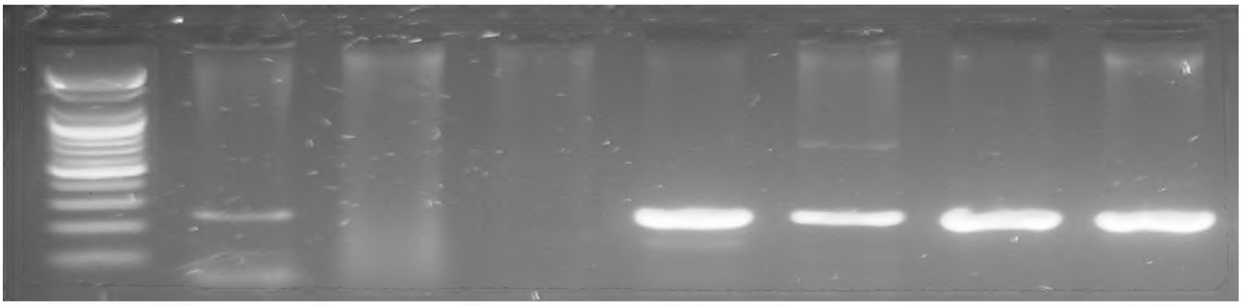

Figure 5: 2% Agarose Gel Electrophoresis of PCR Products of *chuA* Gene (279 bp) Lane 1 Represent Ladder (100 bp), Lane 2-8 Represent *E. cloacae* Isolates

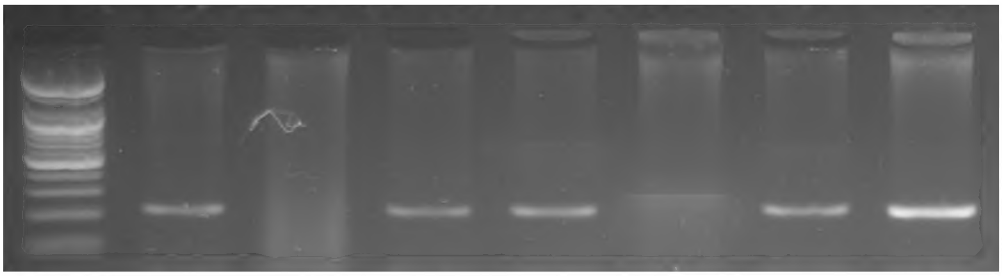

Figure 6: 2% Agarose Gel Electrophoresis of PCR Products of *yjaA* Gene (211 bp) Lane 1 Represent Ladder (100 bp), Lane 2-8 Represent *E. cloacae* Isolates

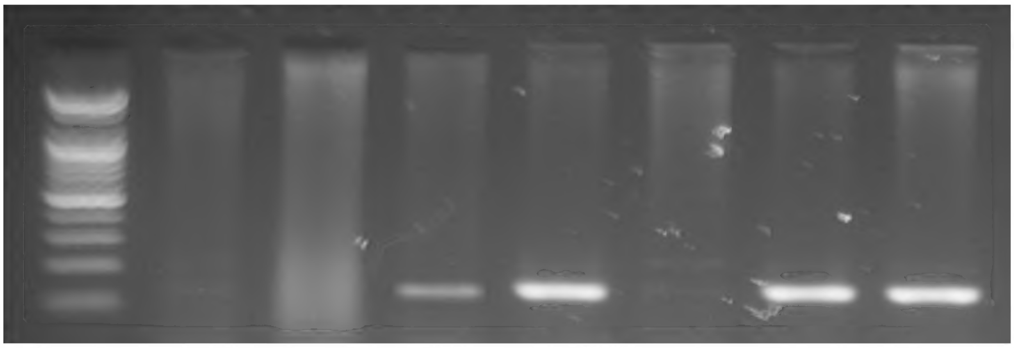

Figure 7: 2% Agarose Gel Electrophoresis of PCR Products of *TspE4C2* Gene (152 bp) Lane 1 Represent Ladder (100 bp), Lane 2-8 Represent *E. cloacae* Isolates

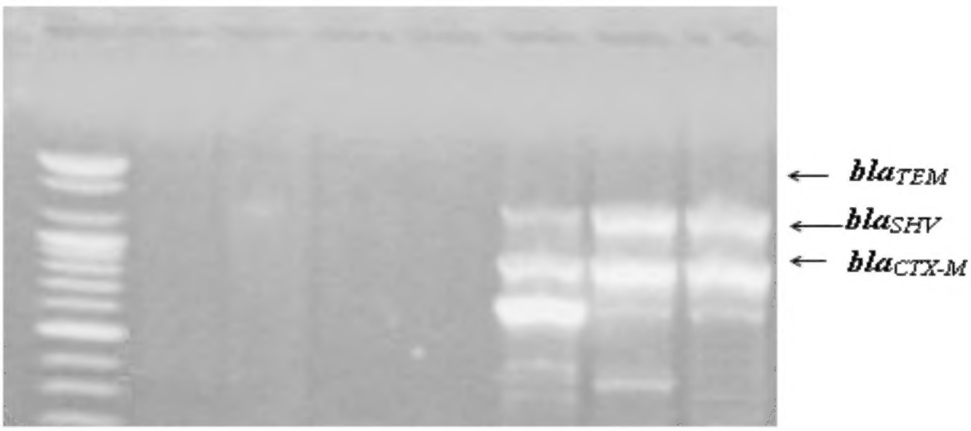

Figure 8: 2% Agarose Gel Electrophoresis of Multiplex PCR Products of bla_{TEM} Gene (1207 bp), bla_{SHV} Gene (730 bp) and $bla_{CTX\text{-}M}$ (550 bp). Lane 1 Represent Ladder (100 bp), Lane 2-8 Represent *E. cloacae* Isolates